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Synthesis of Amino[2.2]paracyclophanes—Beneficial Monomers for Bioactive Coating of Medical Implant Materials**

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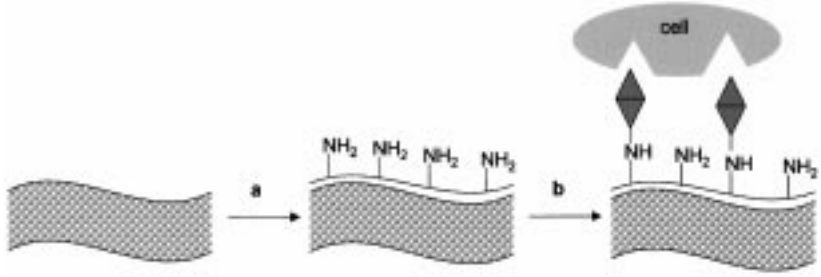
Substituted [2.2]paracyclophanes have received increasing interest over the past few years.[1] Beside their use as chiral auxiliaries for asymmetric synthesis[2] and as ligands in metal clusters,[3] they were reported to be suitable monomers for chemical vapor deposition (CVD) polymerization.[4] CVD-based polymer coatings were of interest as interfaces for biomedical applications due to their potential for the incorporation of functional groups (Scheme 1). These functional groups can be used to conjugate biomolecules such as proteins, antigens, or cell receptors to implant surfaces.[5] The resulting biomimetic coatings provide interfaces that may allow control of the interactions between biomaterials and organisms. There is an increasing demand for bioactive surfaces, particularly in rapidly growing fields such as tissue engineering.[6]



Scheme 1. Concept of bioactive coating for tissue engineering based on CVD polymerization of amino[2.2]paracyclophanes. a) CVD polymerization of amino[2.2]paracyclophane provides a reactive interface. b) Linkage of cytokines to the interface, for example cell receptors, growth factors, antigens, or cell adhesion mediators, controls interactions with cells.

Several substituted [2.2]paracyclophanes were reported to undergo CVD polymerization.[7] Although amino-substituted polymers are interesting polymers for biomedical applications, their synthesis by CVD is limited by the lack of facile and effective syntheses of amino-functionalized [2.2]paracyclophanes. 4-Amino[2.2]paracyclophane (**3a**) is commonly synthesized from **1** by a five-step synthesis[8] via [2.2]paracyclophanecarboxylic acid and Curtius rearrangement. This synthesis provides poor yields of **3a**, and diamino[2.2]paracyclophane (**3b**) has yet to be synthesized following this approach. Alternatively, **3a** was recently prepared from 4-bromo[2.2]paracyclophane by metalation with butyllithium and successive amination in 46% yield.[9] The direct synthesis of amino[2.2]paracyclophanes by nitration and subsequent reduction of the nitro[2.2]paracyclophanes suffers from the poor resistance of [2.2]paracyclophanes to oxidation and its tendency to polymerization. In early studies, Cram et al. reported the synthesis of nitro[2.2]paracyclophanes by treatment of [2.2]paracyclophane with mixtures of nitric acid and sulfuric acid that resulted in yields of 26% for the mononitro compound **2a** and of 8% for the dinitro product **2b**.[10] In addition to poor yields, purification of the products from polymeric by-products was difficult, which limited the broad application of this route. Herein we describe a new convenient high-yield synthesis route to **3a** and **3b**.

Treatment of anhydrous nitric acid with trifluoromethanesulfonic acid delivers free nitronium ions[11] which exhibit high nitration power even at low temperatures (Scheme 2). We found that **1** is completely nitrated at temperatures as low as $-78\,^{\circ}C$. Due to the low temperatures and short reaction times, side reactions like oxidation or polymerization of **1** are not favored and were not observed. As a result, yields of the nitro[2.2]paracyclophane **2b** were increased from 8% to 93%.

These nitration conditions could be adjusted to synthesize selectively either **2a** or **2b**. Using the superacidic ion-exchange resin Nafion/nitric acid, **2a** is obtained in 95% yield. Synthesis of dinitro[2.2]paracyclophane **2b** is best carried out with stirring for 30 min at $-78\,^{\circ}C$ and an additional 2 h at $-20\,^{\circ}C$. Only traces of **2a** were found under these conditions. The main product **2b** (93%) was determined to comprise mainly the pseudo-*para* isomer **2b′** (75%), with about 25% of the pseudo-*meta* isomer (**2b″**). Other isomers were less than 2%, as shown by gas chromatography. This ratio was not affected by the subsequent reduction and is responsible for the fact that **3b** contains 22.7% pseudo-*meta* diamino[2.2]paracyclophane (**3b″**) (Table 1).

Reduction of the nitro[2.2]paracyclophanes was previously carried out with hydrogen using platinum catalysts.[12] However, these reaction conditions are unfavorable and yields are low to moderate. Therefore, more efficient routes are necessary if amino-substituted [2.2]paracyclophanes are to be exploited as potential monomers for CVD. Several

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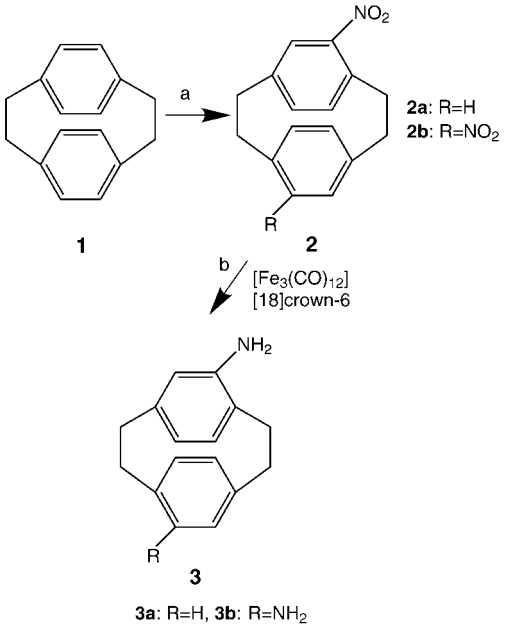

Scheme 2. Synthesis of amino- and diamino[2.2]paracyclophanes **3** (for the disubstituted compounds the pseudo-*meta* isomer is shown). a) Trifluoromethanesulfonic acid/nitric acid (1:1, 100%), dichloromethane, low temperature, or nafion/nitric acid (100%), dichloromethane, room temperature; b) phase-transfer catalysis, toluene/aqueous KOH (1N), room temperature.

Table 1. ^{1}H NMR chemical shifts of aromatic protons in **3a** and **3b**.

	Yield[a] [%]	*ortho*	*meta*	*para*	pseudo-*ortho*	pseudo-*meta*	pseudo-*para*	pseudo-*geminal*
3a	95	5.38	6.18	6.05	6.32	6.32	6.51	7.08
3b′	68.5	5.44	6.17	6.58	6.17	5.44	–	6.58
3b″	22.7	5.44	6.94	6.00	6.00	–	5.44	6.94

[a] Determined by GC/MS; ratio **3b′**/**3b″** is based on GC/MS of **2b′** and **2b″**; other isomers were determined only in traces.

reduction systems such as Zn/HCl, $SnCl_2$/HCl, Sn/HCl, $LiAlH_4$, and $NaBH_4$ were unsuitable as well since reaction did either not occur or yielded the corresponding diazo compounds. Herein, we used a strategy incorporating triiron dodecacarbonyl which is in situ converted to $[Fe_3(CO)_{12}]^{\bullet-}$, a powerful reductant even under mild reaction conditions.[13] Reactions were carried out in toluene/methanol[14] or under organic/aqueous phase-transfer conditions in the presence of a strong base.[15] The use of the two-phase system toluene/aqueous KOH and [18]crown-6 as phase-transfer catalyst led to a straightforward reduction of both **2a** and **2b**. In contrast to the reduction with triiron dodecacarbonyl in homogenous medium,[13] this reaction system does not require heating under reflux. Quantitative reduction of **2a** and **2b** is achieved with stirring for 2 h at room temperature. No polymerization or degradation products of [2.2]paracyclophanes were found under these conditions. Compounds **3a** and **3b** were free of unreacted nitro or diazo compounds as determined by GC/MS, IR, and NMR studies.

The combination of nitration at low temperature in superacidic medium and reduction with $[Fe_3(CO)_{12}]^{\bullet-}$ at room temperature provides an extremely mild pathway toward **3a** and **3b**. Strict control of selectivity and milder reaction conditions allow the synthesis of **3a** and **3b** in overall yields as high as 90%. So far, the direct synthesis of **3b** from [2.2]paracyclophane as a two-step procedure was reported only in two cases.[11, 16] Overall yields for the two-step procedure were either not reported or as low as 4% to 7%.[17]

CVD polymerization of **3a** resulted in transparent polymer films of poly(amino-*para*-xylylene-co-*para*-xylylene) (**4**). The polymer coating shows excellent adhesion on the copper substrates used in this study. Polymer **4** is insoluble in all common solvents. Its elemental composition was determined by X-ray photoelectron spectrocopy (XPS) to be in accordance with the theoretical composition. The IR spectrum of polymer **4** confirmed the presence of primary amino groups as indicated by characteristic signals at wavelengths of 3359 and 3431 cm^{-1}. These free amino groups provide anchor groups for further functionalization. The feasibility of using polymer **4** as functionalized interface for biomedical applications was recently proven by the immobilization of a thrombin inhibitor on metallic cardiovascular implants.[18]

Experimental Section

2a: Acidic ion exchange resin (Nafion) (15.44 g) was incubated in argon atmosphere with nitric acid (100%; 5 mL) for 12 h and subsequently rinsed five times with dichloromethane. [2.2]Paracyclophane (420 mg, 2 mmol) dissolved in dichloromethane (50 mL) was added to a suspension of the superacidic resin in dichloromethane (50 mL). After the mixture had been stirred for 90 min at 0 °C and 60 min at 24 °C, the suspension was quenched with ice water, extracted with diethyl ether, and purified by column chromatography to give **2a** in 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): $\delta = 2.88-3.30$ (7H, CH_2), 4.10 (1H), 6.63–6.95 (4H, CH), 7.23–7.33 (3H, CH); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): $\delta = 33.54$, 34.27, 34.66, 35.34, 126.31, 127.98, 129.21, 135.51, 136.55, 137.18, 137.38, 137.69, 141.68, 142.02, 142.39, 149.83; IR (KBr): $\tilde{\nu} = 868$ (CN), 1196 (NO_2), 1519 (NO_2), 2938 (CH) cm^{-1}; MS (70 eV): *m*/*z*: 253 [M^+], 104 [main, $C_8H_8^+$], 91 [$C_7H_7^+$], 78 [$C_6H_6^+$], 65 [$C_5H_5^+$].

2b: Nitric acid (100%; 0.8 mL) was added at room temperature to a solution of trifluoromethanesulfonic acid (3.5 mL) in dichloromethane (30 mL). After stirring for 15 min under an argon atmosphere, the solution was cooled to −78 °C and a solution of [2.2]paracyclophane (2 g, 9.6 mmol) in dichloromethane (80 mL) was added slowly. The solution was stirred for 20 min at −78 °C and another 120 min at −20 °C. Quenching with ice water, extraction with diethyl ether, and subsequent column chromatography delivered **2b** in 93% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): $\delta = 2.88-3.30$ (7H, CH_2), 4.10 (1H), 6.63–6.95 (3H, CH), 7.23–7.33 (3H, CH); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): $\delta = 33.54$, 34.27, 34.66, 35.34, 126.31, 127.98, 129.21, 135.51, 136.55, 137.18, 137.38, 137.69, 141.68, 142.02, 142.39, 149.83; IR (KBr): $\tilde{\nu} = 868$ (CN), 1196 (NO_2), 1519 (NO_2), 2938 (CH) cm^{-1}; MS (70 eV): *m*/*z*: 298 [M^+], 149 [$C_8H_7-NO_2^+$], 133[$C_8H_7-NO^+$], 103 [$C_8H_7^+$], 91 [main, $C_7H_7^+$], 77 [$C_6H_5^+$], 65 [$C_5H_5^+$].

3a: 4-Nitro[2.2]paracyclophane (2 g, 6.67 mmol), triiron dodecacarbonyl (1.12 g, 6.67 mmol) and [18]crown-6 ether (90 mg) were dissolved in toluene (200 mL). Subsequently, 1N KOH (200 mL) were added and the solution was stirred for 2 h at room temperature. Extraction with diethyl ether and subsequent column chromatography delivered **3a** in 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): $\delta = 2.54-2.62$ (1H, CH_2), 2.64–3.08 (7H, CH_2), 3.36 (2H, NH_2), for aromatic protons see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$, TMS): $\delta = 32.23$, 33.03, 34.95, 35.37, 122.36, 122.99, 124.60, 126.82, 131.48, 132.43, 133.23, 133.44, 138.91, 138.97, 141.06, 144.72; IR (KBr): $\tilde{\nu} = 722$, 800, 1287, 1425, 1509, 1562, 1620, 2857, 2928, 3002, 3032, 3061, 3383, 3474 cm^{-1}; MS (70 eV): *m*/*z*: 223 [M^+], 119 [main, $C_8H_7NH_2^+$], 104 [$C_8H_8^+$], 91 [$C_7H_7^+$], 78 [$C_6H_6^+$], 65 [$C_5H_5^+$].

3b: Dinitro[2.2]paracyclophane (2 g, 6.67 mmol), triiron dodecacarbonyl (2.24 g, 13.23 mmol), and [18]crown-6 ether (176 mg) were allowed to react and worked-up as described for **3a**. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): $\delta =$

2.54 – 2.62 (1H, CH_2), 2.64 – 3.05 (6H, CH_2), 3.08 – 3.19 (1H, CH_2), 3.36 (4H, NH_2), for aromatic protons see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$, TMS): $\delta = 29.42, 31.48, 32.60, 34.55, 120.29, 120.46, 122.06, 122.26, 123.69, 123.83, 128.44, 133.76, 140.32, 140.36, 144.91, 145.45$; IR (KBr): $\tilde{\nu} = 675, 726, 803, 1429, 1509, 1562, 1620, 2857, 2928, 3002, 3222, 3336, 3360, 3430\ cm^{-1}$; MS (70 eV): m/z: 238 [M^+], 119 [main, $C_8H_7NH_2^+$], 91 [$C_7H_7^+$], 77 [$C_6H_5^+$], 65 [$C_5H_5^+$].

4-Amino[2.2]paracyclophane (**3a**) was polymerized by using a self-designed CVD installation consisting of a sublimation zone, a pyrolysis zone, and a deposition chamber equipped with a sample holder.[7] Compound **3a** (30 mg) was placed in the sublimation zone and a copper substrate was fixed on the sample holder being cooled to 8 °C. The pressure was adjusted to 0.1 mbar and the pyrolysis zone was heated to 750 °C. Subsequently, **3a** was sublimed slowly resulting in a transparent film of polymer **4** on the copper substrate. XPS (referenced to hydrocarbon at 285.0 eV): C_{1s}: 93.9 % (calcd: 94.1 %), N_{1s}: 6.1 % (calcd: 5.9 %) atom %; IR (grazing angle of 85°): $\tilde{\nu} = 814, 865, 1155, 1286, 1424, 1516, 1583, 1618, 2863, 2946, 3012, 3047, 3359, 3431\ cm^{-1}$.

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Active-Site Structure and Dynamics of Cytochrome *c* Immobilized on Self-Assembled Monolayers—A Time-Resolved Surface Enhanced Resonance Raman Spectroscopic Study**

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Self-assembled monolayers (SAM) of alkanethiol derivatives on metal electrodes provide numerous possibilities for immobilizing redox proteins.[1] In the past few years, such devices have gained increasing interest in (nano)biotechnology. In particular, the current and potential importance of immobilized enzymes for the design and development of biosensors,[2] bioelectronic systems,[3] or biocatalytic cells[4] has stimulated a large number of experimental studies in this field. Customizing the catalytic functions and optimizing the efficiencies of these systems represent a challenge in current research as they require a detailed characterization of the structural and functional properties of the redox-active adlayers as well as of the dynamics of the processes involved. Such investigations impose high demands on the sensitivity and selectivity of the analytical tools. In most cases, microscopic and electrochemical methods such as atomic force microscopy and cyclic voltammetry (CV) are employed which, however, do not provide information about the molecular structure of the species involved in the redox process. In this respect, time-resolved surface-enhanced resonance Raman (TR-SERR) spectroscopy, which exclusively probes the vibrational spectra of the redox sites solely of the immobilized species, is a powerful alternative approach for analyzing molecular structure and dynamics of the adsorbed enzymes.[5]

So far, TR-SERR spectroscopy was restricted to redox proteins immobilized on bare Ag electrodes which, however, do not represent systems of general biotechnological applicability due to potential denaturation of proteins directly adsorbed on the Ag surface. In this work, we have employed TR-SERR spectroscopy for the first time to probe the heterogeneous electron transfer (ET) process of a heme protein immobilized on a SAM-coated Ag electrode. As a test redox protein we chose cytochrome *c* (Cyt-*c*) which has been studied by various electrochemical techniques including CV, electrochemical impedance, and electroreflectance.[6–8]

After electrochemcial roughening of the Ag electrode,[5] SAMs of 11-mercaptounadecanoic acid (11-MUDA; Aldrich) were prepared according to previously published procedures.[9] For the adsorption of Cyt-*c*, the electrode was dipped in a solution containing 2 μM of the purifed protein (horse heart, Sigma) as well as 12.5 mM KCl and 12.5 mM phosphate buffer (pH 7.0) for 30 min. Subsequently, this

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